

RESEARCH PAPER

Co-ordination of hydraulic and stomatal conductances across light qualities in cucumber leaves

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Abstract

Long-term effects of light quality on leaf hydraulic conductance (K_{leaf}) and stomatal conductance (g_s) were studied in cucumber, and their joint impact on leaf photosynthesis in response to osmotic-induced water stress was assessed. Plants were grown under low intensity monochromatic red (R, 640 nm), blue (B, 420 nm) or combined red and blue (R:B, 70:30) light. K_{leaf} and g_s were much lower in leaves that developed without blue light. Differences in g_s were caused by differences in stomatal aperture and stomatal density, of which the latter was largely due to differences in epidermal cell size and hardly due to stomatal development. Net photosynthesis (A_N) was lowest in R-, intermediate in B-, and highest in RB- grown leaves. The low A_N in R-grown leaves correlated with a low leaf internal CO_2 concentration and reduced PSII operating efficiency. In response to osmotic stress, all leaves showed similar degrees of stomatal closure, but the reduction in A_N was larger in R- than in B- and RB-grown leaves. This was probably due to damage of the photosynthetic apparatus, which only occurred in R-grown leaves. The present study shows the co-ordination of K_{leaf} and g_s across different light qualities, while the presence of blue in the light spectrum seems to drive both K_{leaf} and g_s towards high, sun-type leaf values, as was previously reported for maximal photosynthetic capacity and leaf morphology. The present results suggest the involvement of blue light receptors in the usually harmonized development of leaf characteristics related to water relations and photosynthesis under different light environments.

Key words: Amphistomatous, *Cucumis sativus*, leaf development, leaf hydraulic conductance, light quality, osmotic stress, photosynthesis, stomatal conductance, stomatal density, stomatal opening.

Introduction

Leaves are a major bottleneck in the plant water transport capacity (Sack and Holbrook, 2006). At least 25% of the whole-plant resistance can be attributed to the leaf hydraulic resistance (i.e. the reciprocal of leaf conductance; $1/K_{\text{leaf}}$), which is disproportional to the relatively short water-transport distance in the leaves (Sack *et al.*, 2003). Efficient water delivery (i.e. high K_{leaf}) maintains leaves well-hydrated. Decreases in K_{leaf} usually cause leaves to become less hydrated, which is often associated with a reduction in stomatal conductance (g_s) and, consequently, in carbon gain (Sperry, 2000; Sack and Holbrook, 2006; Johnson *et al.*, 2009). Such a stomatal

response is necessary for leaves to avoid damaging water potentials and runaway cavitation events (Sperry *et al.*, 2002). It is thought that, in the long-term, the economics of gas exchange will set limits on the hydraulic capacity of leaves. Since the synthesis of xylem tissue is costly, the construction costs for the plant would be such that do not exceed the benefits of photosynthetic performance (Brodribb *et al.*, 2010). It can therefore be expected that there is co-ordination between the capacity for liquid water transport through the leaf, the capacity for gas transport between the leaf and the surrounding air (water and CO_2), and the leaf photosynthetic capacity.

Abbreviations: A_N , net leaf photosynthesis; B, blue light; C_i , intercellular CO_2 concentration; ECD, epidermal cell density; g_s , stomatal conductance; K_{leaf} , leaf hydraulic conductance; LED, light emitting diode; PPFD, photosynthetic photon flux density; R, red light; RB, red and blue light (R:B, 70:30); SD, stomatal density; SI, stomatal index; Φ_{PSII} , photosystem II operating efficiency; Ψ_π , osmotic potential.

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Several studies have shown that liquid phase conductance (i.e. K_{leaf}) and vapour phase conductance (i.e. g_s) are strongly co-ordinated both in short- (Sperry *et al.*, 1993; Brodribb and Jordan, 2008) and the long-term (Brodribb *et al.*, 2005; Sack *et al.*, 2005; Lo Gullo *et al.*, 2010). An increase in water supply capacity, by a higher K_{leaf} , would only benefit photosynthesis in the presence of sufficiently high intercellular CO_2 . g_s is determined by the cross-sectional area available for gas fluxes, i.e. the product of pore area per stoma and stomatal density (Parlange and Waggoner, 1970). Short-term tuning of g_s takes place by adjustments in the stomatal pore aperture (stomatal opening), while long-term adaptations of g_s are mostly due to changes in the stomatal density and size (determining maximum g_s ; Franks and Beerling, 2009).

Besides density and size of stomata, their distribution among the two leaf surfaces might also influence gas exchange (Foster and Smith, 1986). Amphistomatous leaves (i.e. stomata at both leaf sides) have both higher water vapour (due to higher boundary layer conductance; Parkhurst, 1978; Mott *et al.*, 1982) and intercellular carbon dioxide (due to thicker mesophyll; Parkhurst and Mott, 1990) conductances. However, amphistomatous leaves can also behave as functionally hypostomatous, by closing or nearly closing the adaxial stomata (Reich, 1984).

Light intensity has been shown to affect the efficiency of water delivery and the photosynthetic performance of leaves. Traits such as high photosynthetic capacity, high K_{leaf} , and high g_s were co-found in sunny environments compared with shaded habitats within and across species (reviewed in Sack and Holbrook, 2006). Recent studies show that light intensity can also influence xylem hydraulic conductances in the rest of the water transport path between roots and leaves (Nardini *et al.*, 2010; Sellin *et al.*, 2010). In natural environments, within plant canopies, differences in light intensity often coexist with differences in spectral quality, as, for example in the case of shade by neighbours (Smith, 1982; Combes *et al.*, 2000). It has been shown in cucumber plants, which were grown under different combinations of red and blue light supplied by light-emitting diodes (LEDs), that light quality by itself can induce photosynthetic and morphological properties in leaves that normally occur at high light intensity, although the plants were grown under low light intensity (Hogewoning *et al.*, 2010b). Considering the often observed coordination between K_{leaf} , g_s , and photosynthetic capacity, one could expect that light quality is also involved in determining the hydraulic transport capacity in leaves, and that light quality might even play a key role in establishing this co-ordination. To the best of our knowledge, the long-term effects of light quality on K_{leaf} in relation to g_s and leaf photosynthetic performance were not previously investigated. Also, no in-depth study has been published on the impact of light quality during leaf development on the ability of leaves to cope and recover from a shortage of water supply with respect to their photosynthetic performance. This information will provide new insights into the ability of plants to optimally tune their multifactorial function across light qualities.

The aims of this study were: (i) to assess if the capacities of the aqueous and gaseous leaf water transport phases and leaf photosynthesis change and remain co-ordinated when plants are grown under different combinations of red and blue light at low light intensity; (ii) to relate g_s with underlying leaf stomatal traits as being influenced by light quality and the leaf amphistomatous character; and (iii) to investigate the plasticity of leaves with different, light quality-induced K_{leaf}/g_s combinations, to short-term water stress.

Cucumber was used as a model plant, since it is an amphistomatous species, has large petiolated simple leaves (facilitating the K_{leaf} measurements), and has already shown differences in photosynthetic capacity and leaf morphology due to the spectral composition of light during growth under low light intensity.

Materials and methods

Plant material and growth conditions

Cucumber seeds (*Cucumis sativus* L. cv. Hoffmann giganta) were sown, germinated, and seedlings were grown up to the cotyledon stage (i.e. fully open cotyledons and before the appearance of the first leaf) under $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD; determined using LI-190/LI-1400, Li-Cor, Lincoln, NE) and 16/8 h day/night cycle by white fluorescent tubes (Philips TLD 58W/84, Eindhoven, The Netherlands). This period was about one week. Subsequently, four seedlings were transplanted in each of the three growth-units ($1 \times w \times h = 0.8 \times 0.8 \times 1.6$ m; Homebox S, EastSide- Impex., Berlin, Germany) under the same PPFD-level but three different light spectral qualities, using red LEDs (R; Red, LXX2-PD12-S00, Philips), blue LEDs (B; Royal-Blue, LXX2-PR14-R00, Philips), and a combination of red and blue LEDs (RB; R:B, 70:30). Light spectra (Fig. 1) were determined with a calibrated spectroradiometer (USB2000 spectrometer, Ocean Optics, Duiven, The Netherlands). The growth-units had a constant day and night temperature (21 ± 1 °C), and relative air humidity (75%) resulting in a vapour pressure deficit of 0.62 ± 0.04 kPa. The plants were grown under $\sim 400 \mu\text{mol mol}^{-1}$ CO_2 concentration. Leaf temperature did not differ significantly between plants grown

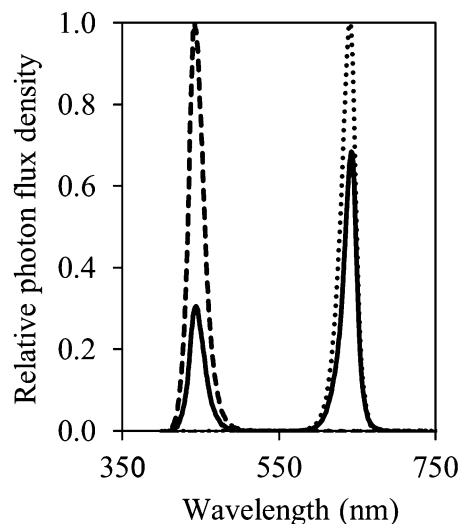


Fig. 1. Light spectra of the red (dotted line), blue (dashed line), and red and blue (70:30; solid line) light environments measured in the growth-units at plant level.

in different light qualities [determined by using an infrared thermometer (Raynger™ ST, Raytec Corporation, Santa Cruz, CA)]. Plants were watered automatically by half-strength Hoagland solution ($\text{pH}=5.4\pm0.2$, $\text{EC}=1\text{ mS cm}^{-1}$) circulating in a well-aerated root dipping hydroponic system. The solution was replenished weekly to sustain the pH and EC at constant levels. Three weeks after transplanting the measurements started on the first fully expanded leaf.

Leaf gas exchange

The measurements of leaf gas exchange [stomatal conductance (g_s), net photosynthetic rate (A_N)], and operating efficiency of photosystem II ($\Phi_{\text{PSII}} = \frac{F_s}{F_m}$; Baker, 2008) were performed using two portable gas exchange systems Li-Cor 6400 (Li-Cor Biosciences, Lincoln, NE) combined with two leaf chamber fluorometers 6400-40 (Li-Cor Biosciences, Lincoln, NE). Microclimatic conditions were adjusted in the leaf chamber to be identical with those of the growth environment. Light intensity in the leaf chamber was set at $100\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ in all cases, while spectral quality was set according to the spectral quality in the growth-unit (treatment). Leaf chamber temperature was stable at $21\text{ }^\circ\text{C}$ and the CO_2 reference concentration was $400\text{ }\mu\text{mol mol}^{-1}$. Relative air humidity was controlled at 75%. All measurements were carried out inside the growth-units. In total, eight plants were used for gas exchange measurements per light quality treatment (2×4 plants per treatment).

Leaf hydraulic conductance (K_{leaf})

The night before the K_{leaf} measurement, polyurethane glue (Kompi power, BISON, Goes, The Netherlands) was applied on the attached petioles of five leaves per light quality treatment and, subsequently, it was surrounded by a silicon tube used as a matrix. This aimed to provide a hard sheath to the fragile petiole, which would withstand compression without damaging the petiole (facilitating connection with the measurement system). The following day, the silicon tube was removed from the petiole and the leaf was excised from the mother plant. All manipulations in the laboratory were done under water to prevent the entrance of air into the vessels that had been opened by cutting. The petiole was adjusted with a razor blade to 2 cm length. A new razor blade was used for each cut. A silicone tube was pushed over the petiole cut end and attached to the measurement system. During measurements, flow was always in the natural direction, from the petiole to the inner leaf compartments. The time between collection from the plant and the start of the measurement was approximately 10 min.

K_{leaf} was measured using the vacuum pump method (Sack *et al.*, 2002). The leaf was sealed inside a glass desiccator with a moist paper towel to maintain a saturated humidity environment, with the tube of solution attached to the petiole running out through the centre of a rubber stopper to a balance. The water was pulled through the leaf using a vacuum pump (7550-62, Barnant, Barrington, IL), while measuring flow rates of solution into the petiole with a balance. Weight was measured at a per second sample rate and averaged over 30 s. A degassed aqueous solution of potassium chloride (10 mM) and calcium chloride (1 mM) at room temperature ($20\pm2\text{ }^\circ\text{C}$) was used throughout the measurements (van Ieperen and van Gelder, 2006). For each leaf, five levels of partial vacuum were applied, 0.04, 0.05, 0.06, 0.07 and 0.08 MPa, in increasing order of absolute air pressure. When the first vacuum level was applied, the flow typically increased for 20 ± 10 min before stabilizing, while after each subsequent vacuum level change, values stabilized typically after 10 ± 5 min. All measurements were made with the desiccator being under the same light source (fluorescent tubes, PPFD: $5\pm2\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$).

The absolute (i.e. non-normalized) hydraulic conductance was calculated by the slope of the regression of flow rate into the leaf versus pressure (equation 1; Kolb *et al.*, 1996; Sack *et al.*, 2002). K_{leaf} was then determined by dividing the absolute conductance of the leaf by its area (determined by ImageJ open access software,

version 1.44p). The method used was independent of the stomatal component. During the measurement, water flow through the lamina was driven by both the suction produced by the pump and the transpiration from the leaf inside the desiccator. The latter was minimized by increasing the humidity in the desiccator with wet tissue paper. Nonetheless, transpiration into the flask created a consistent driving force at different vacuum levels, i.e. it affected only the y-intercept, and not the slope of the flow versus vacuum level relationship. The reason is that transpiration rate is a function of the mole fraction driving force and g_s , neither of which is affected by ambient pressure (Nobel, 1991).

$$\text{abs}K_{\text{leaf}} = \frac{\Delta\text{flow rate}}{\Delta\text{vacuum level}} \quad (1)$$

Stomatal traits

All morphological stomatal features were determined using the silicon rubber impression technique (Weyers and Meidner, 1990). Details of the impression method and image analysis are described by Fanourakis *et al.* (2011). The measurements were made on four leaves (one leaf per plant) per light quality treatment on both leaf surfaces. Stomatal features were assessed at both adaxial (i.e. upper) and abaxial (i.e. lower) leaf epidermes, using a part of the leaf that was midway between the tip and the base and away from the edge. Leaf veins were avoided as they support no stomata, and all cell densities quoted are therefore interveinal values. For determining the stomatal and epidermal cell densities (SD and ECS, respectively) a magnification of $\times250$ was used, and five fields of view were counted in each sampling area. Rectangular fields of view (Poole and Kurschner, 1999) were used and an unbiased decision was made concerning the inclusion of stomata and non-stomatal epidermal cells on the edge of a field of view (Kubinova, 1993). Stomatal index (SI) was calculated according to Salisbury (1928). ECD was estimated as the guard cell density ($2\times\text{SD}$) plus the non-stomatal epidermal cell density. For the individual stomatal parameters, a magnification of $\times1000$ was used, and 20 randomly selected stomata per sampling area were measured. The stomata length, stomata width, pore length, and pore aperture were measured (Fig. 2). Stoma width was chosen instead of guard cell width, since the latter undergoes changes up to 50% as stomata close (Shope and Mott, 2006). To calculate pore area, it was assumed that the pore was elliptical (major axis pore length; minor axis pore aperture). The same procedure was used for estimating stomatal area (major axis stomatal length, minor axis stomatal width). Pore area per leaf area was calculated as the sum of the pore area per stoma \times stomatal density of the two leaf surfaces.

Effect of short-term osmotic stress on leaf gas exchange and dynamics of recovery

To estimate the effect of short-term osmotic stress applied to the roots on the photosynthetic performance of fully developed leaves, as well as their recovery when stress application ceases, two levels of osmotic potential were realized in the growth medium (0.10 and 0.15 MPa) of four plants per light quality treatment. The osmotic potential of the nutrient solution was initially 0.05 MPa (control), and was adjusted by adding PEG-6000 (Michel and Kaufmann, 1973). The osmolality of the solutions was measured (using VAPRO 5520, Wescor inc., Logan, UT), and was then converted to osmotic potential (Ψ_π) using the van't Hoff equation (Nobel, 1991). The g_s , A_N , intercellular CO_2 concentration (C_i), and Φ_{PSII} were measured on leaves of plants developed under the different light qualities, as described in gas exchange measurements. Following the measurements, the control medium was replenished by another medium with an osmotic potential of 0.10 MPa. The plants were left in this medium for the next 24 h. Subsequently, the above-mentioned parameters were recorded. The same procedure was followed for the second level of osmotic stress, where the nutrient

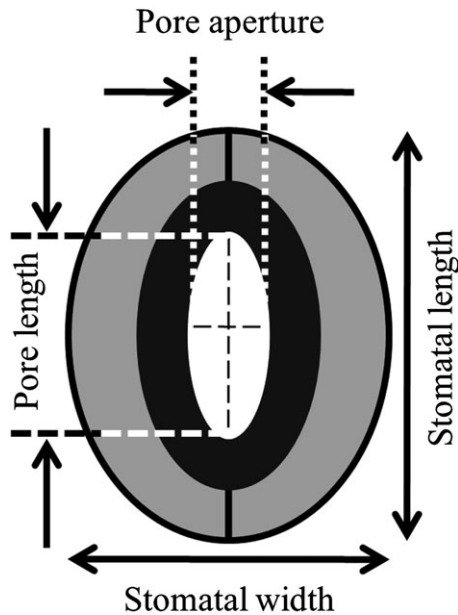


Fig. 2. Schematic representation of the stomatal and pore dimensions as measured by image analysis. The grey area represents the guard cells, the black area the pore walls, and the white area (internal ellipse) depicts the stomatal pore area.

solution was replaced by the one having the highest osmotic potential (0.15 MPa). Plants were left for 24 h and then were measured. Finally, the plants were returned to the control medium (0.05 MPa) and left to recover. Similar measurements were performed during the next 24 and 48 h.

Data analysis

The data were analysed using the statistical analysis software package IBM SPSS Statistics 19 (IBM Corporation, NY). The parameters investigated were statistically tested by one-way analysis of variance (ANOVA) and the means were compared by the least significant difference (LSD) multiple comparison test ($P \leq 0.05$). The effects of light quality on leaf stomatal traits were tested on both leaf sides (abaxial and adaxial) and statistically tested by two-way ANOVA. Linear regression analysis ($P \leq 0.05$) was used to test the significance of the correlation between K_{leaf} and g_s , between K_{leaf} and Φ_{PSII} and between K_{leaf} and A_N of leaves developed under the three different light qualities investigated in this study.

Results

Leaf gas exchange and hydraulic conductance (K_{leaf})

Light quality during leaf development exerted a considerable influence on both gas exchange parameters and K_{leaf} (Fig. 3). The absence of blue light (R) resulted in very low g_s and K_{leaf} values. g_s and K_{leaf} of leaves developed under B and RB light were at least 3-fold higher compared with leaves developed under R light (Fig. 3A, B). A_N was the highest in leaves which developed under RB light, and the lowest in R-grown leaves (Fig. 3C). Φ_{PSII} was significantly lower (15%) in R-grown than in RB- and B-grown leaves, which had approximately similar Φ_{PSII} (about 0.74; Fig. 3D). g_s showed a strong and statistically significant correlation

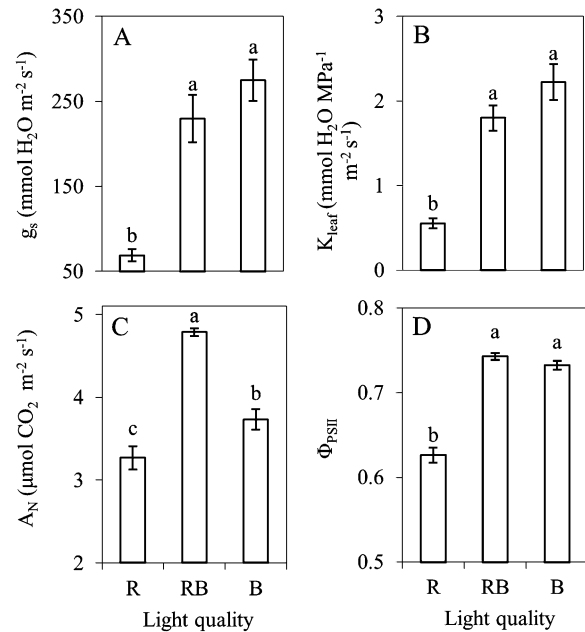


Fig. 3. Stomatal conductance (g_s , A), leaf hydraulic conductance (K_{leaf} , B), net photosynthetic rate (A_N , C), and operating efficiency of PSII (Φ_{PSII} , D) of cucumber leaves, grown at different light qualities [R, red; RB, red and blue (red:blue, 70:30); and B, blue]. Different letters indicate significant differences ($P \leq 0.05$; $n=5$ for K_{leaf} , $n=8$ for the other parameters). Error bars indicate the SEM.

with K_{leaf} across the applied light qualities ($r^2=1.0$; $P < 0.05$; Fig. 4), although it has to be considered that the regression analysis is done on the averages obtained from only three light quality treatments. No statistically significant correlations were observed between Φ_{PSII} and K_{leaf} , and between A_N and K_{leaf} across the applied light qualities.

Stomatal traits

Light quality significantly affected almost all the examined stomatal anatomical features that can contribute to g_s . Leaves grown in the presence of blue light (B and RB) had significantly higher adaxial, abaxial, and total (adaxial + abaxial) SDs compared with R-grown leaves (Table 1). In all treatments, adaxial SD was lower than abaxial SD. The fraction adaxial over the total SD was similar (0.39) on leaves developed in the presence of blue light and slightly reduced (0.32) on leaves of R-grown plants. Differences in SI directly reflect differences in the percentage of epidermal cells that undertook differentiation to stomatal cells during leaf development. SI was significantly lower on the adaxial leaf surface than on the abaxial leaf surface at all light qualities. Light quality influenced SI only on the adaxial leaf surface, where the SI was slightly lower on R-grown leaves than on B- and RB-grown leaves (Table 1). ECD was not different between the adaxial and abaxial leaf surfaces, while R-grown leaves had a significantly lower ECD on both leaf surfaces than leaves that were grown in the presence of blue light (Table 1). The combination of larger epidermal cells (i.e. lower ECD) and similar (abaxial) or

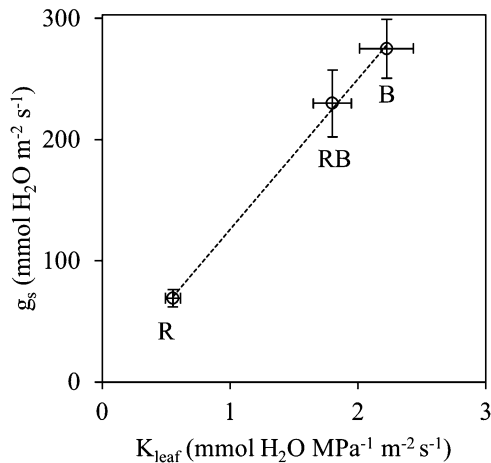


Fig. 4. Relationship of mean stomatal conductance (g_s , $n=8$) to mean leaf hydraulic conductance (K_{leaf} , $n=5$) across the three investigated light qualities [R, red; RB, red and blue (red:blue, 70:30); and B, blue]. A significant linear regression ($r^2=1$, $P<0.05$) suggests a proportional relation between g_s and K_{leaf} . Error bars indicate the SEM.

slightly reduced (adaxial) differentiation percentage into stomata (i.e. similar or lower SI) explains the reduced SD on R-grown leaves compared with RB- and B-grown leaves (Table 1). Stomatal size, represented by stomatal area, was not different between the adaxial and abaxial leaf surfaces and between the light qualities, despite a small but statistically significant effect of both factors on stomatal width (Table 1). Pore aperture of individual stomata was significantly higher on the abaxial than on the adaxial leaf surface, and higher in B-grown leaves than in R-grown leaves (Table 1). As expected, the pore area per leaf area (i.e. sum of pore area per stoma \times SD of the two leaf surfaces) was not evenly distributed between the adaxial and abaxial leaf surfaces, and was mostly situated ($\sim 70\%$) at the abaxial leaf surface in all light qualities investigated (Table 1). Leaves, which developed in the presence of blue light had a significantly higher pore area per leaf area than R-grown leaves, with B-grown leaves having more than double the pore area per leaf area than R-grown leaves (Table 1). The differences observed were due to both differences in stomatal density and pore aperture.

Effect of short-term osmotic stress on leaf gas exchange and dynamics of recovery

The induction of osmotic water stress in the root environment, in two steps on consecutive days from -0.05 through -0.10 to -0.15 MPa, increasingly reduced g_s in leaves grown at all light qualities (Fig. 5A). Despite the large differences in absolute reductions of g_s among the light quality treatments, relative reductions in g_s were comparable among the leaves grown at the different light qualities, and similarly increased with increasing osmotic stress (Fig. 5B). During the first 48 h after release from the osmotic stress, similar recovery patterns of relative g_s were observed among all leaves, irrespective of

Table 1. Stomatal traits on the adaxial and abaxial surfaces of cucumber leaves grown at different light qualities [R, red; B, blue; RB, red and blue (red:blue, 70:30)].

Different letters indicate significant differences ($P \leq 0.05$; $n=4$). SD, stomatal density; ECD, epidermal cell density; SI, stomatal index.

Stomatal trait	Light quality			Mean
	R	RB	B	
SD (no. mm⁻²)				
Adaxial	153	285	273	237 b
Abaxial	322	438	417	393 a
Mean	238 b	361 a	345 a	
Total	475 b	723 a	690 a	
SI (–)				
Adaxial	0.10 c	0.14 b	0.14 b	0.13
Abaxial	0.24 a	0.26 a	0.24 a	0.25
Mean	0.17	0.20	0.19	
ECD (no. mm⁻²)				
Adaxial	1744	2216	2145	2035
Abaxial	1686	2152	2122	1987
Mean	1715 b	2184 a	2133 a	
Stomatal length (μm)				
Adaxial	21.0	21.6	22.8	21.8
Abaxial	22.1	21.0	21.0	21.3
Mean	21.5	21.3	21.9	
Stomatal width (μm)				
Adaxial	11.8	11.9	12.9	12.2 b
Abaxial	12.6	13.2	13.6	13.1 a
Mean	12.2 b	12.6 b	13.2 a	
Stomatal area (μm²)				
Adaxial	194	202	231	209
Abaxial	218	218	224	220
Mean	206	210	228	
Pore length (μm)				
Adaxial	12.1 bc	13.6 a	12.7 ab	12.8
Abaxial	12.7 ab	11.0 c	11.2 c	11.6
Mean	12.4	12.3	12.0	
Pore aperture (μm)				
Adaxial	0.81	0.61	0.90	0.78 b
Abaxial	0.82	1.24	1.55	1.20 a
Mean	0.82 b	0.93 ab	1.23 a	
Pore area per leaf area (μm² mm⁻²)				
Adaxial	1210	1849	2448	1836 b
Abaxial	2642	4644	5579	4288 a
Mean	1926 b	3247 a	4013 a	
Total	3852 b	6493 a	8027 a	

light quality (Fig. 5B). Before applying osmotic stress, C_i was already significantly lower in R-grown leaves than in leaves grown in the presence of blue light (Fig. 5F). Neither the application of osmotic stress, nor the release from osmotic stress induced a statistically significant change in C_i in the R-grown leaves (Fig. 5F). In the leaves grown in the presence of blue light, a very clear reduction and recovery of C_i was observed, but only at the highest osmotic stress (Fig. 5F). In all leaves, A_N was reduced in response to the highest level of osmotic stress, but this reduction was more severe in R-grown leaves than in the RB- and B-grown leaves. In RB- and B-grown leaves, full recovery of A_N appeared within 24 h after the release from osmotic stress, but not in R-grown

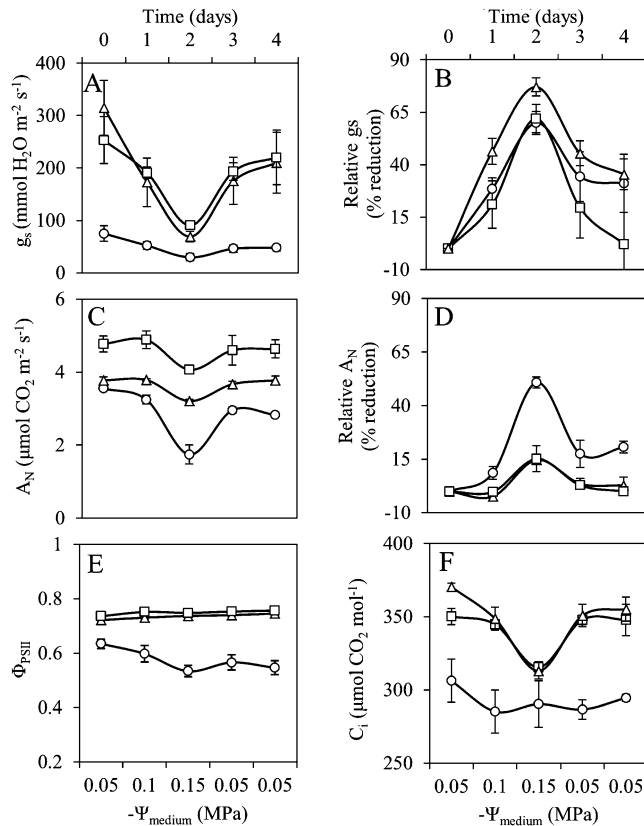


Fig. 5. Stomatal conductance (g_s , A), net photosynthetic rate (A_N , C), operating efficiency of PSII (Φ_{PSII} , E), and intercellular CO_2 concentration (C_i , F) of cucumber leaves, grown at the three investigated light qualities (circles, R-grown; triangles, B-grown; squares, RB-grown leaves) and at constant growth medium osmotic potential (Ψ_π , 0.05 MPa), which were further transferred to different growth medium Ψ_π values. Time 0 corresponds to measurements at the control medium prior to transfer. All measurements were conducted 1 d after each respective change of the growth medium Ψ_π . (B) and (D) depict the relative reduction of the g_s and A_N , respectively. Values are the means of measurements in four leaves \pm SEM.

leaves, which still suffered some reduction in A_N at the end of the experiment (48 h after release of the stress). Φ_{PSII} only decreased in R-grown plants after the application of osmotic stress and did not recover after alleviating osmotic stress (Fig. 5E).

Discussion

Co-ordination of K_{leaf} and g_s under monochromatic red, blue, and combined red and blue light

In this study, it has been shown for the first time that both K_{leaf} and g_s are largely influenced by light quality during leaf development, but that the co-ordination between K_{leaf} and g_s remains conserved (Fig. 4). Differences in both K_{leaf} and g_s due to monochromatic red, blue or combined red and blue light during leaf development were large (up to 3-fold), and revealed that both g_s and K_{leaf} were largely enhanced by the

presence of blue light (Fig. 3A, B). Increased g_s and K_{leaf} were also observed in response to a high light intensity (Sellin *et al.*, 2008) and were related to optimal functioning under high light conditions. Supplying light at an intensity of only $100 \mu mol m^{-2} s^{-1}$ for $16 h d^{-1}$ is sufficient to grow normal cucumber plants, as was previously shown under different light spectra, including the solar spectrum (Hogewoning *et al.*, 2010a) and combinations of only red and blue light (Hogewoning *et al.*, 2010b). The latter research showed that adding and increasing the proportion of blue in a red/blue light spectrum at low light intensity increased maximal photosynthetic capacity and leaf mass per area (Hogewoning *et al.*, 2010b), a parameter reflecting leaf morphological changes in response to light intensity towards ‘sun-type’ leaves (Poorter *et al.*, 2009). It is shown here that K_{leaf} and g_s increase in a co-ordinated way in response to the presence of blue light in the light spectrum (Fig. 3A, B). This provides indirect evidence for the involvement of blue light receptors (cryptochromes, phototropins) in the long-term regulation of K_{leaf} and g_s and further strengthens the idea that blue light stimulates ‘sun-type’ characteristics in leaves (Hogewoning *et al.*, 2010b).

The effect of light quality on K_{leaf}

Light intensity influences leaf hydraulics via effects on K_{leaf} , as has been shown in shade- and sun-adapted leaves of many angiosperms (Nardini *et al.*, 2005; Sellin and Kupper, 2007). So far, the role of light quality during leaf development on K_{leaf} has received less attention. Leaf hydraulic conductance is composed of an intra- and an extra-xylem component connected in series, with the latter possibly having the lowest conductivity (i.e. highest resistance) in amphistomatous leaves (Mott, 2007). A higher conductance of leaf xylem is mainly related to wider xylem conduits and higher venation densities, while the conductance of the extra-xylem pathway is influenced by a palisade/spongy mesophyll ratio and leaf thickness (Sack and Holbrook, 2006), as well as by environmental factors, which can fluctuate in the short-term (i.e. light intensity and temperature) via the regulation of plasma membrane aquaporins (Cochard *et al.*, 2007). It has been observed in *Capsicum annuum* that, besides secondary xylem thickness in stems, the thickness of palisade and spongy mesophyll in leaves was enhanced in light spectra containing blue rather than monochromatic red light (Schuerger *et al.*, 1997). Recent studies also suggested that short-term changes in light intensity (Scoffoni *et al.*, 2008) and quality (Voicu *et al.*, 2008; Sellin *et al.*, 2011) affect K_{leaf} through changes in the conductance of the extra-xylem component. For instance, a short-term ($\leq 5 h$) light treatment of *Betula pendula* shoots resulted in the lowest K_{leaf} values under red light, intermediate under white, and highest under blue light (Sellin *et al.*, 2011). However, it is most likely that, in the present study, the observed differences in K_{leaf} due to light quality can be mainly attributed to structural changes that occurred during leaf development because all measurements of K_{leaf} were done at the same light spectrum and intensity

(fluorescent tubes, PPFD: $5 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$). The latter strengthens the notion that light quality during leaf development modulates K_{leaf} through structural changes, but supplementary research is necessary to find out if this is caused by structural changes in either the intra- or extra-xylem water transport pathway in the leaf.

The effect of light quality on g_s and underlying stomatal traits

In the present study, it is shown in cucumber that monochromatic red light during leaf development strongly reduced g_s compared with monochromatic blue or a combination of blue and red light. An analysis of the explanatory factors of g_s reveals that differences in both pore aperture and SD play a role and together cause a strong effect on pore area per leaf area (Table 1). The relative contribution of the abaxial surface to leaf g_s was similar for the three light qualities investigated ($\sim 70\%$; Table 1), demonstrating that the amphistomatous character of the cucumber leaves was unaffected by light quality. SD was significantly higher on leaves grown in the presence of blue light (Table 1; Wang *et al.*, 2009; Hogewoning *et al.*, 2010b). This higher SD was not due to an increased production of stomata, as SI was not substantially affected by the presence of blue light (Table 1). Instead, this higher SD was due to a higher ECD (i.e. a lower epidermal cell size) on leaves that developed in the presence of blue light (Table 1). The present results show that stomatal pore aperture increases with an increasing percentage of blue light, but it remains unclear whether this is caused by structurally larger stomata (Table 1), or by a direct effect of blue light on stomatal opening. Many previous studies demonstrated that blue light significantly enhances stomatal conductance via a rapid and reversible regulation of stomatal aperture (Meidner, 1968), probably mediated by the blue-light-absorbing carotenoid zeaxanthin (Zeiger *et al.*, 2002), but also phototropins and cryptochromes (Kinoshita *et al.*, 2001; Mao *et al.*, 2005) might play a role. Almost no studies have been devoted on the specific role of blue light on stomatal development. Schoch *et al.* (1984) demonstrated in *Vigna sinensis* that supplying blue light during the night decreased SI, whereas red light applied at the same period increased SI. In *Dendranthema grandiflorum* an increasing fraction of blue slightly increased SI (Rajapakse and Kelly, 1993) and in *Gossypium barbadense* an increasing blue/red ratio increased adaxial SD (Lu *et al.*, 1993). Recently, Kang *et al.* (2009) showed, in *Arabidopsis thaliana*, that blue light via cryptochrome (CRY) inhibits the production of COP1, a key repressor of photomorphogenesis, which resulted in a stimulation of stomatal development. Similar effects were shown for phytochrome (PHY) B (Lake *et al.*, 2001), while in *Arabidopsis thaliana* the effects of CRY, phyA, and phyB were shown to be additive (Kang *et al.*, 2009). The effects of osmotic stress in the root environment on g_s demonstrated that functional stomata developed under all the applied light qualities (Fig. 5A, B). The almost similar relative reduction in g_s , caused by osmotic stress in all the applied light qualities, indicates similar degrees of stomatal closure. The large

differences in absolute g_s between light quality treatments were most likely due to different SDs, thus mostly a result of structural rather than functional differences. Previously, it has been reported that g_s of cucumber leaves, which developed under monochromatic red light, was unresponsive to both light intensity and quality (Hogewoning *et al.*, 2010b). In this study, it has been demonstrated that the stomata on R-grown leaves kept normal responsiveness to the induction and release from osmotic stress (Fig. 5). This indicates at least partial functioning of the stomata in response to hormonal (e.g. abscisic acid) and hydraulic signals (Comstock, 2002). It also suggests that light and drought-related signals might influence g_s in an independent manner (Harada and Shimazaki, 2009).

Photosynthetic plasticity under osmotic stress of leaves that differ in K_{leaf} due to light quality

Based on an extensive study of 16 forest species, which yielded a strong correlation between K_{leaf} and Φ_{PSII} , it has been suggested that the maximum photosynthetic rate of the leaves is constrained by their vascular supply (Brodribb and Feild, 2000). It is shown here that the absence of blue light causes a substantial reduction in both K_{leaf} and Φ_{PSII} (Fig. 3B, D). However, the presence of blue and red light enhances net photosynthesis when compared with the monochromatic light qualities (Fig. 3C). The latter was probably caused by specific effects of the light spectrum on photosynthetic performance. Hogewoning *et al.* (2010b) indicated that the lower A_N in B-grown leaves is related to a lower chlorophyll content compared with RB-grown leaves, while the lower quantum efficiency of photosynthesis of blue compared with red light (McCree, 1972) and a suboptimal distribution of photons over PSII and PSI due to light quality (Hogewoning *et al.*, 2010b) could also have contributed to the observed differences in photosynthetic rates. The lower A_N in R-grown leaves compared with B- and RB-grown leaves, which is paralleled by reductions in both Φ_{PSII} and C_i (Fig. 5E, F), seems to suggest stomatal limitation on photosynthesis. However, variations in mesophyll conductance for CO_2 transport in the leaf, caused by differences in light quality, also cannot be excluded. It has previously been suggested that blue light might influence mesophyll conductance in the short-term (Loreto and Tsonev, 2009). However, Hogewoning *et al.* (2010b) had already shown that, most likely, the low photosynthetic rates and Φ_{PSII} observed in R-grown leaves were due to disorders in the development and functioning of the photosynthetic machinery, as sustained by reduced values of dark-adapted F_v/F_m and gas exchange measurements under low O_2 . The additional reduction of Φ_{PSII} during osmotic stress, which was only observed in R-grown leaves and remained after the release from the stress, indicates further damage of the photosynthetic machinery. These results demonstrate that the leaves, which developed under monochromatic red light, were more vulnerable to leaf damage induced by osmotic stress than the B- and RB-grown leaves. Although this reduction in photosynthetic performance of R-grown leaves correlates well with

a low K_{leaf} and a low C_i compared with the other light quality treatments, it provides no evidence for a causal relationship between a limitation in leaf water supply and the reduced ability to deal with osmotic induced stress.

Conclusions

In cucumber, light quality during leaf development strongly influences K_{leaf} as well as g_s , but K_{leaf} and g_s remain co-ordinated across growth-light qualities. Light quality structurally influences leaf g_s via an effect of epidermal cell size on stomatal density. The absence of blue light causes a severe reduction in both the water supply/demand capacities and leaf photosynthetic performance, while the photosynthetic performance of R-grown leaves was shown to be highly vulnerable to water stress. Based on our results, it seems likely that the presence of blue light during growth plays a critical role in optimally tuning the multifactorial function of leaves.

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